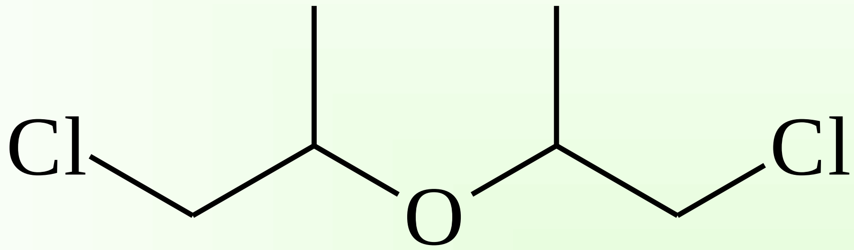
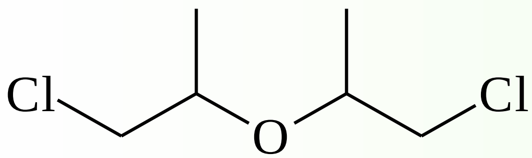
Technical Grade Bis(2-chloro-1-methylethyl) Ether

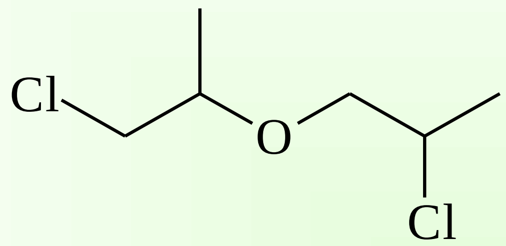


Molecular Weight: 171.07 CAS Registry No.: NA

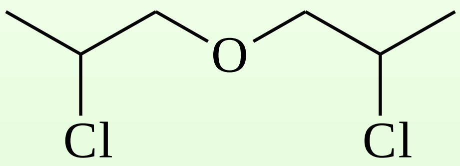
Technical Grade BCMEE



BCMEE



2-Chloro-1-methylethyl (2-chloropropyl) ether



Bis(2-chloro-*n*-propyl) ether

BCMEE Use/Occurrence

- By-product of propylene glycol and propylene oxide manufacture
- Solvent in paint and varnish removers, spotting agents
- Intermediate in dye synthesis
- Active ingredient in the nematocide Nemamorte® (Japan)

Carcinogenicity of BCMEE

- Carcinogenicity in humans:
  - No data

- Carcinogenicity in animals:
  - Oral gavage studies in mice (NTP, 1982)
  - Oral gavage studies in rats (NCI, 1979)
  - Dietary studies in mice (Mitsumori *et al.*, 1979)

Tumors in Male Mice (NTP, 1982)

Tumor Site and Type		**Dose, mg/kg$_{bw}$**		
		0	100	200
Males				
Lung: Alveolar/ Bronchiolar	Adenoma	5/50	13/50*	11/50
	Carcinoma	1/50	2/50	2/50
	Adenoma or carcinoma	6/50	15/50*	13/50
Liver	Adenoma	8/50	10/50	13/50
	Carcinoma	5/50	13/50*	17/50*
	Adenoma or carcinoma	13/50	23/50*	27/50*
	Metastases to lung	1/50	4/50	3/50
Stomach / Forestomach	Squamous-cell papilloma	0/49	1/50	1/50

* Significant increase above controls ($p < 0.05$ by Fisher Exact test).

Tumors in Female Mice (NTP, 1982)

Tumor Site and Type		**Dose, mg/kg$_{bw}$**		
		0	100	200
Females				
Lung: Alveolar/ Bronchiolar	Adenoma	1/50	4/50	8/50*
	Carcinoma	0/50	0/50	2/50
	Adenoma or carcinoma	1/50	4/50	10/50*
Stomach / Forestomach	Squamous-cell papilloma	0/50	0/49	2/49
	Squamous-cell carcinoma	0/50	0/49	1/49

* Significant increase above controls ($p < 0.05$ by Fisher Exact test).

Non-positive Findings

- Rat oral gavage studies (NCI, 1979)
- Mouse dietary studies (Mitsumori *et al.*, 1979)

Genotoxicity of BCMEE

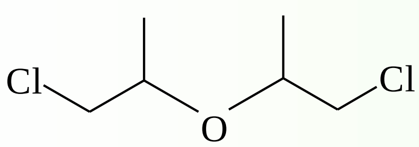
- Bacterial assays:
  - Mixed findings in *Salmonella* reverse mutation assays (with and without metabolic activation)
  - Non-positive findings in *Escherichia coli*

Genotoxicity of BCMEE (cont.)

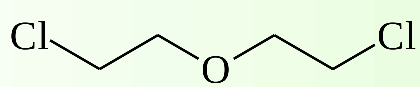
- Mammalian cell assays
  - Positive in mouse lymphoma forward mutation assay without metabolic activation
  - Positive for chromosomal aberrations (+S9) and SCE (+/-S9) in CHO cells
  - Positive for S-phase synthesis in mouse hepatocytes; Negative UDS

Structure-Activity Comparisons

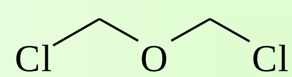
- Carcinogenicity of other haloethers:
  - bis(chloroethyl) ether (BCEE)
  - bis(chloromethyl) ether (BCME)
  - chloromethyl methyl ether (CMME)



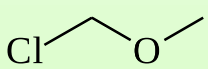
BCMEE



BCEE



BCME

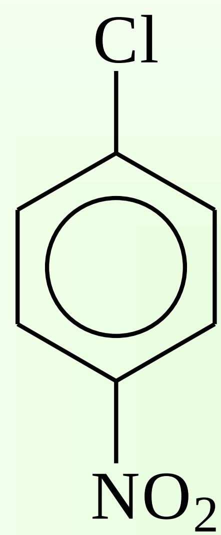


CMME

BCMEE: Summary

- Animal evidence for carcinogenicity:
  - Induction of liver tumors in male mice
  - Induction of lung tumors in male and female mice
  - Some rare forestomach tumors in mice
- Other relevant evidence
  - Genotoxicity, structure-activity analogies

Evidence of the Carcinogenicity of 1-Chloro-4-nitrobenzene



Molecular Weight: 157.56   CAS Registry No.: 100-00-5

Carcinogenicity Studies of 1-Chloro-4-nitrobenzene

Tumor incidence in HaM/ICR mice administered 1-chloro-4-nitrobenzene in feed for 18 months (Weisburger *et al.*, 1978).

Tumor Type	**Dose (ppm in feed)**			
	0 (simultaneous)	0 (pooled)	3,000	6,000
Males				
Hepatocellular carcinomas	1/14 *(7%)*	7/99 *(7%)*	4/14[a] *(29%)*	0/14 *(0%)*
Vascular tumors	0/14 *(0%)*	5/99 *(5%)*	2/14 *(14%)*	4/14[a] *(29%)*
Females				
Vascular tumors	0/15 *(0%)*	9/102 *(9%)*	3/20 *(15%)*	7/18[a] *(39%)*

[a] Different from pooled controls ($p<0.05$)

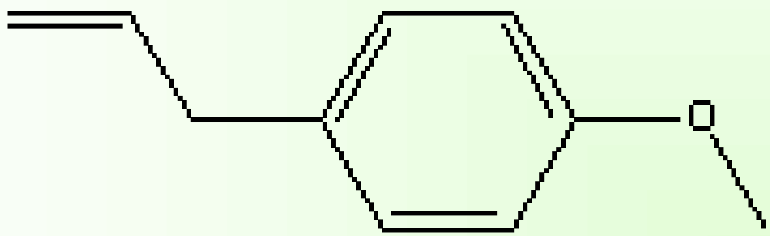
Other Relevant Data

- Produced mutations in some, but not the majority, of tests in *Salmonella*
- Produced DNA strand breaks *in vitro* and *in vivo*
- Produced sister chromatid exchanges and chromosomal aberrations *in vitro*
- Metabolized to the carcinogen, 4-chloroaniline

Summary: 1-Chloro-4-nitrobenzene

- Vascular tumors in male and female mice
- Hepatocellular tumors at the lower of two doses in male mice
- Genotoxic effects in mammalian cells *in vitro* and *in vivo*
- Metabolism to a known carcinogen

Evidence of the Carcinogenicity of Estragole



Molecular Weight: 148.20   CAS Registry No.: 140-67-0

Use, Production and Occurrence

- Estragole is used for its flavor and fragrant properties in numerous food products, drinks, perfumes, cosmetics, soaps and detergents.
- Production
  - U.S. TSCA >1 million pounds in 1990
  - OECD “high production volume” chemical in 1997
- Major component (30 to 75 %) of volatile oils of anise, basil, bay, tarragon, and other herbs
- Minor component of oils of fennel, marjoram, and chervil, oil of turpentine, and tobacco smoke

Carcinogenicity Studies of Estragole

- Humans
  - No evidence available
- Animals (Drinkwater et al., 1976; Miller et al., 1983; Wiseman et al., 1987)
  - Eight cancer bioassays
  - CD-1, $B6C3F_1$, and A/J mice
  - Oral, i.p., and s.c. administration

Carcinogenicity Studies of Estragole

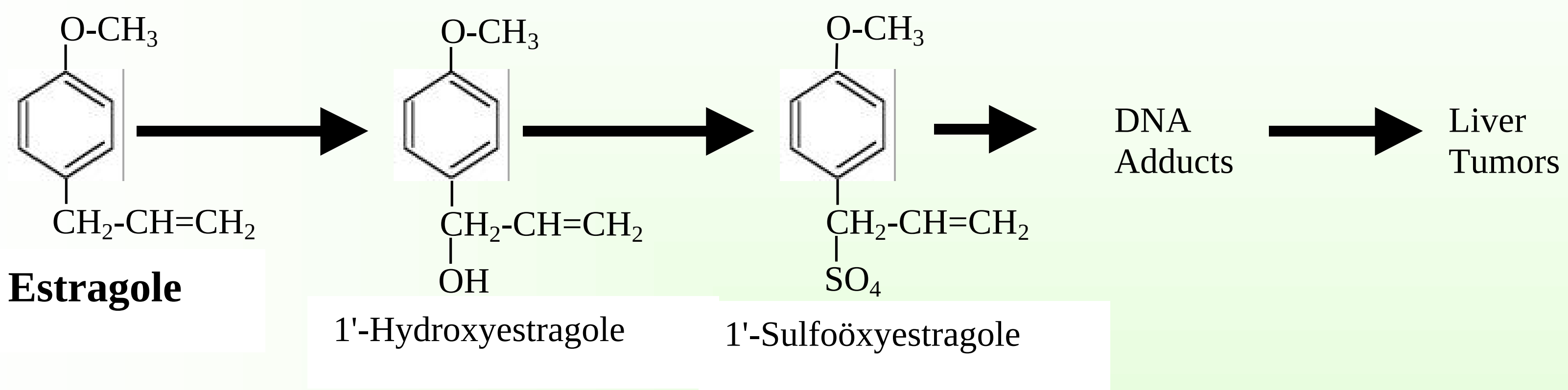
Route of exposure Test animal	Treatment	Sacrifice (months)	Result[a] (liver tumors)
oral			
Male newborn CD-1 mice	10 gavage doses	14	+ ($p<0.001$)
Female newborn CD-1 mice	10 gavage doses	14	- ($p=0.16$)
Female CD-1 mice	diet 12 months, 2 dose groups	20	+ ($p<0.001$) + dose-response
i.p.			
Male newborn CD-1 mice	4 doses	12	+ ($p<0.001$)
Male newborn $B6C3F_1$ mice	4 doses	18	+ ($p<0.001$)
Male newborn $B6C3F_1$ mice	1 dose	10	+ ($p<0.001$)
Female A/J mice	24 doses	8	- (lung tumors)
s.c.			
Male newborn CD-1 mice	4 doses, 2 dose groups	15	+ ($p<0.05$) + dose-response

[a] Incidence of hepatocellular carcinoma relative to vehicle controls, except in the A/J mouse study which assayed only for lung tumors.

Carcinogenicity Studies of 1'-Hydroxyestragole, the Putative Toxic Metabolite of Estragole

- 1'-Hydroxyestragole induced high incidences of liver tumors in mice (Drinkwater *et al.*, 1976; Miller *et al.*, 1983; Wiseman *et al.*, 1987)
  - diet for 12 months to adult female CD-1 mice
  - i.p. newborn male CD-1, $B6C3F_1$, CeH/HeJ, or C57Bl/6J mice
  - s.c. newborn male CD-1 mice
- No increases in tumors in rats given 20 s.c. injections and sacrificed at 24 months

Carcinogenic Mode of Action



- Mechanism is the same as safrole (a Prop. 65 listed carcinogen)
- Six equivalent DNA adducts characterized for estragole and safrole
- Inhibition of the sulfation step significantly reduces DNA adduct formation and prevents liver tumor formation.

Other Relevant Data -- Genotoxicity

- Reverse mutations in *Salmonella*: mixed results for estragole and 1'-hydroxyestragole
- UDS in rat hepatocytes: positive for estragole and 1'-hydroxyestragole
- UDS in human cell lines: positive for estragole and 1'-hydroxyestragole
- DNA adducts and abasic sites observed
- DNA adduct levels in mice in vivo of different alkenylbenzene compounds, including estragole, correlated well with liver tumor incidences

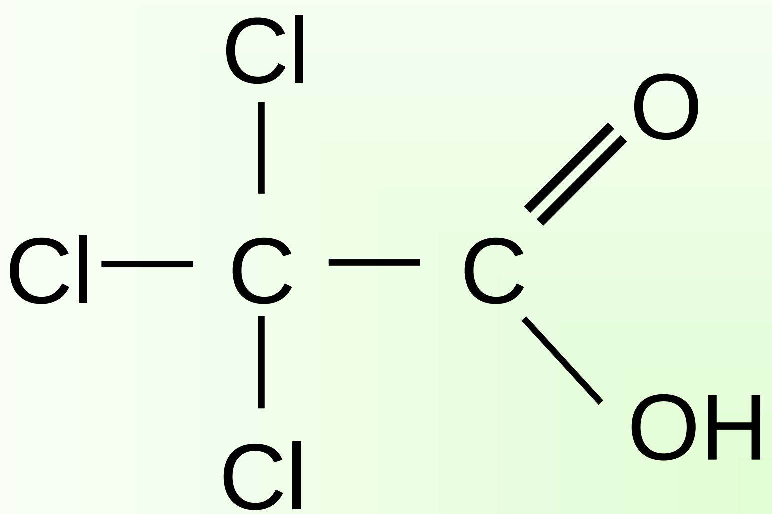
Other Relevant Data -- SAR

- Structural similarities to other many alkenylbenzene compounds observed to be carcinogenic
  - safrole, 1'-hydroxysafole
  - methyleugenol, 1'-hydroxymethyleugenol
  - others: *cis*-asarone, *trans*-asarone, 1'-hydroxy-2',3'-dehydroestragole, 1'-acetoxyestragole, 1'-hydroxy-2',3'-dehydrosafrole, 1'-acetoxysafrole, 1'-hydroxyelemicin, and 1'-acetoxyelemicin

Summary

- Estragole induced liver cancer in multiple strains and both sexes of mice exposed by several different routes of administration.
- Genotoxicity
- Chemical-structural analogies with recognized carcinogens
- Relatively clear understanding of the carcinogenic mode of action

Trichloroacetic Acid (“TCA”)



Molecular Weight = 163.39 CAS Registry No. 76-03-9

TCA use/occurence

- Synthetic intermediate
- Minor uses: medication, reagent
- Former use: selective herbicide (principally as the Na^+ salt). The most recent registration was cancelled in 1992
- TCA is one of the major by-products of the disinfection of water by chlorination

TCA occurrence (ii)

- Concentrations measured in U.S. drinking water supplies in one study ranged from 4 to 103 μg/L
- Formed (with other chloroacetic acids, halomethanes *etc*.) by reaction of Cl_2 or hypochlorite with organic substances, *e.g.* humic acid.
- TCA is also found in other situations where water is chlorinated, such as irrigation, swimming pools, and pulp mill effluents.

Carcinogenicity of TCA

- Carcinogenicity in humans:
  - No data
- Carcinogenicity in animals:
  - A number of bioassays have been reported
  - TCA is a hepatocarcinogen in the mouse. The male is more sensitive than the female.
  - In a single rat study, TCA was hepatotoxic but not hepatocarcinogenic.

Carcinogenicity Studies of TCA

Route	Species	Strain	Sex	Tumor site, type	IARC eval.?	Authors
oral (drinking water)	Mouse	$B6C3F_1$	M	hepatocellular adenoma (ad.) and carcinoma (ca.)	yes	Herren-Freund *et al.*, 1987
oral (drinking water)	Mouse	$B6C3F_1$	M, F	hepatocellular ca. in males only	yes	Bull *et al.*, 1990
oral (drinking water) #1	Mouse	$B6C3F_1$	M	hepatocellular ad. and ca.	no	DeAngelo and Daniel, 1990; DeAngelo, 1991
oral (drinking water) #2	Mouse	$B6C3F_1$	M	hepatocellular ad. and ca.	no	
oral (drinking water)	Mouse	$B6C3F_1$	F	hepatocellular ad. and ca.	no	
oral (drinking water)	Mouse	$B6C3F_1$	F	hepatocellular ad. and ca.	no	Pereira, 1996
oral (drinking water)	Mouse	$B6C3F_1$	F	hepatocellular ca.	no	Pereira and Phelps, 1996.
oral (drinking water)	Rat	F344	M	No increases in tumor incidence	no	DeAngelo and Daniel, 1992; DeAngelo, 1991; De Angelo *et al.*, 1997.

Hepatocellular Tumors in male B6C3F$_1$ mice receiving ENU and/or TCA

Herren-Freund *et al.* (1987)

Treatment		*Result*					
ENU, mg/kg	TCA, mg/L	N	Mice with Adenomas	Adenomas / mouse	Mice with Carcinomas	Carcinomas/ mouse
10	5	28	11 (39%)	0.61±0.16	15 (54%)	0.93±0.22
2.5	5	23	**6 (26%)**	**0.30±0.12**	**11 (48%)**	**0.57±0.21**
2.5	2	33	**11 (33%)**	**0.42±0.12**	**16 (48%)**	**0.64±0.14**
0	5	22	**8 (36%)**	**0.50±0.16**	**7 (32%)**	**0.50±0.17**
10	0	23	9 (39%)	0.52±0.15	9 (39%)	0.57±0.20
2.5	0	22	1 (5%)	0.05±0.05	1 (5%)	0.05±0.05
0	0	22	2 (9%)	0.09±0.06	0 (0%)	0

Significantly different from control (P < 0.01 by Fisher's exact test):
Carcinogenic effect **Tumor promoting effect**

Hepatocellular lesions in male $B6C3F_1$ mice receiving TCA in drinking water

Bull *et al.* (1990)

Treatment			***Result: Number of lesions (number of mice)***					
					Diagnosis of lesions:			
TCA, g/L	Duration (weeks)	N	Total lesions	Lesions examined	Normal	Hyper-plastic	Adenoma	Carcin-oma
2	52	24	**30 (19[b])**	16 (11)	1 (1)	10 (9)	1 (1)	4 (4)
2	37	11	**5 (4[a])**	5 (4)	0	2 (2)	0	3 (3)
1	52	11	**7 (5[b])**	7 (5)	0	3 (1)	2 (2)	2 (2)
0	-	35	2 (2)	2 (2)	1 (1)	1 (1)	0	0

Significantly increased ([a] $P < 0.05$, [b] $P<0.01$) relative to control, by Fisher's Exact Test.

Hepatocellular tumors in $B6C3F_1$ mice receiving TCA in drinking water

DeAngelo and Daniel (1990); DeAngelo (1991)

- Experiment 1: Male mice; 0, 0.05, 0.5 or 5 g TCA/L drinking water (0, 8, 71 and 595 mg/kg bw/day) for 60 weeks.
  - Hepatocellular adenomas + carcinomas increased in mice receiving 0.5 (37.9%) and 5 g TCA/L (55.2%), compared to controls (13.3%)
  - Not significantly increased in mice receiving 0.05 g/L TCA.
- Experiment 2: Male mice; 0 or 4.5 g TCA/L drinking water (0 and 583 mg/kg bw/day) for 94 weeks.
  - Hepatocellular tumors increased in exposed (86.7%) *vs.* controls (15%).
- Experiment 3: Female mice; 0, 0.5 or 4.5 g TCA/L drinking water (0, 71 and 583 mg/kg bw/day) for 104 weeks.
  - Hepatocellular tumors (ad. and ca.) increased in mice receiving 4.5 g TCA/L (60%) compared to controls (7.7%).
  - Not significantly increased in mice receiving 0.5 g TCA/L.

Hepatocellular lesions in female B6C3F$_1$ mice receiving TCA in drinking water

Pereira (1996)

Treatment		***Incidence of lesions: Number of animals (percentage of animals)***			
TCA, mM	Duration (days)	N	Foci of altered hepatocytes	Hepato-cellular Adenoma	Hepato-cellular Carcinoma
20	360	20	0	2 (10)	**5 (26.3)**
	576	18	**11 (61.1)**	**7 (38.9)**	**5 (27.8)**
6.67	360	19	0	3 (15.8)	0
	576	27	**9 (33.3)**	3 (11.1)	**5 (18.5)**
2.0	360	40	3 (7.5)	3 (7.5)	0
	576	53	10 (18.9)	4 (7.6)	0
0	360	40	0	1 (2.5)	0
	576	90	10 (11.1)	2 (2.2)	2 (2.2)

Significantly increased ($P<0.01$) relative to control, by Fisher's Exact Test.

Hepatocellular lesions in female B6C3F$_1$ mice receiving TCA in drinking water

Pereira and Phelps (1996)

Treat-ment	Mean number of lesions per mouse ± standard error (percentage incidence)						
	31 weeks			***52 weeks***			
TCA mM	N[b]	Foci / mouse	Adenomas / mouse	N	Foci / mouse	Adenomas / mouse	Carcinomas / mouse
20	10	0 (0)	0 (0)	19 +1	0 (0)	0.15±0.11 (10)	0.5±0.18 [e] (25)
6.67	10	0 (0)	0 (0)	19	0 (0)	0.21±0.12 (15.8)	0 (0)
2.0	15	0 (0)	0 (0)	40	0.08±0.04 (7.5)	0.08±0.04 (7.5)	0 (0)
0	15	0.13±0.13 (6.7)	0.13±0.13	40	0 (0)	0.03±0.03 (2.5)	0 (0)

Significantly different from control group by Mann-Whitney test: $P < 0.05$.

Male Fischer 344 rats receiving TCA in drinking water

DeAngelo and Daniel (1992); DeAngelo (1991); DeAngelo *et al*. (1997)

- Male rats; 0.0, 0.05, 0.5 or 5 g TCA/L drinking water (0, 3.6, 36 and 378 mg/kg bw/day) for 104 weeks.
  - No significant increase in hepatocellular tumors in exposed rats.

Tumor initiation/promotion studies

All Studies:
TCA Route = Oral (drinking water)

Initiator	Species	Strain	Sex	End point	Result	Authors
ENU	Mouse	$B6C3F_1$	M	hepatocellular tumors	Carcinogenicity +ve, promotion -ve	Herren-Freund *et al.*, 1987
MNU	Mouse	$B6C3F_1$	F	Liver tumors & foci (eosinophilic, basophilic)	Carcinogenicity +ve, promotion +ve	Pereira and Phelps, 1996
MNU	Mouse	$B6C3F_1$	F	Liver tumors & foci (eosinophilic, basophilic)	Promotion +ve	Pereira et al., 1997
DEN, Partial Hepatectomy	Rat	Sprague-Dawley	M	γGT positive liver foci	Promotion +ve	Parnell *et al.*, 1988

Carcinogenicity Studies of TCA: Results

- Mice:
  - Multiple independent studies in a single strain ($B6C3F_1$).
  - Liver adenoma and carcinoma.
  - All studies positive.
  - Both sexes.
- Rats:
  - Single study.
  - No carcinogenic effect observed.

Genotoxicity of TCA:
standard assays

- Bacterial Mutagenicity:
  - mostly negative.
- Mammalian cells in vitro:
  - very weak: pH effect?
- Mammals in vivo: chromosomal effects
  - micronuclei (inconsistent, high dose only?), aberrations, sperm abnormalities.

Genotoxicity of TCA:
oncogene & DNA effects

- DNA strand breaks.
  - Some positives: mice more sensitive than rats.
- Oxidative DNA damage.
  - Weak positive or negative results: inconsistent.
- Effects on proto-oncogenes & oncoproteins.
  - Consistent changes in tumors: different from DCA.
- DNA Synthesis.
  - Increases in mice associated with cell proliferation (not repair).

Structure-Activity Comparisons

- Other chlorinated acetic acids:
  - Dichloroacetic acid causes liver cancer in mice
  - Monochloroacetic acid not carcinogenic to mice or rats, but severe toxicity might mask response
- Other chlorinated aliphatic compounds:
  - TCE and PCE (of which TCA is a metabolite) are identified as carcinogens for the purposes of Proposition 65.

Mechanism: Alternatives proposed (i)

- Genotoxic / DNA reactive?
  - For:
    - Some clastogenic effects
    - DNA strand breakage and oxidative damage.
  - Against:
    - Most genotoxicity results negative: the few “positives” are equivocal or inconsistent.
    - TCA not intrinsically reactive.
    - No evidence of metabolism to a reactive intermediate.
  - Conclusion:
    - Probably not.

Mechanism: Alternatives proposed (ii)

"Non-genotoxic" (*i.e.* not DNA reactive):

- Peroxisome proliferation (PP)?
  - For:
    - Observed in rodents exposed to TCA and DCA.
    - More marked in mice than rats.
  - Against:
    - Not a large effect, even in mice.
    - Compare DCA and TCA: PP similar, but tumorigenic effects, oncogene activation different.
    - Reports of DNA oxidative damage not substantiated.
  - Conclusion:
    - PP occurs, but its role in TCA carcinogenesis (if any) is unclear.

Mechanism: Alternatives proposed (iii)

- Enhanced cell proliferation due to cytotoxicity
  - For:
    - Proliferation observed in mice
  - Against:
    - Probably not sufficient alone to explain tumor formation.
    - Cause or effect?
- Other growth regulatory effects
  - For/Against:
    - Maybe: insufficient detail to evaluate.
- Overall Conclusion: Insufficient information to determine and characterize mechanism.

Trichloroacetic acid: Summary.

- Animal evidence for carcinogenicity:
  - Positive in both sexes of one strain of the mouse, in multiple experiments.
  - Tumor promoter in rat and mouse liver.
  - negative in rat (1 study).
- Weak (much negative or equivocal) evidence of genetic toxicity.
- Mechanistic arguments against human relevance, but no clear proof of mechanism(s).